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Review

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Mechanisms of the Wnt Pathways as a Potential Target Pathway in Atherosclerosis

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ABSTRACT

The proteins of the Wnt family are involved in a variety of physiological processes by means of several canonical and noncanonical signaling pathways. Wnt signaling has been recently identified as a major player in atherogenesis. In this review, we summarize the existing knowledge on the influence of various components of the Wnt signaling pathways on the initiation and progression of atherosclerosis and associated conditions. We used the PubMed database to search for recent papers on the involvement of the Wnt pathways in atherosclerosis. We used the combination of "Wnt" and "atherosclerosis" keywords to find the initial papers, and chose papers published after 2018. In the first section of the paper, we describe the general mechanisms of the Wnt signaling pathways and their components. The next section is dedicated to existing studies assessing the implication of Wnt signaling elements in different atherogenic processes, such as cholesterol retention, endothelial dysfunction, vascular inflammation, and atherosclerotic calcification of the vessels. Lastly, various therapeutic strategies based on interference with the Wnt signaling pathways are considered. We also compare the efficacy and availability of the proposed treatment methods. Wnt signaling can be considered a potential target in the treatment and prevention of atherosclerosis. Therefore, in this review, we reviewed evidences showing that wnt signaling is an important signal for developing appropriate treatment strategies for atherosclerosis.

Keywords: Wnt signaling; Atherosclerosis; Cardiovascular disease

OVERVIEW OF THE Wnt PATHWAYS

The term Wnt is a portmanteau of wingless—a critical segmentation gene in *Drosophila*—and int-1, a homologous gene in mammals. This protein family includes 19 glycoproteins that attach to the receptors of the Frizzled family (Fzd). Ten variants of Fzd can be observed in humans, and the corresponding proteins have seven transmembrane domains recognized as a separate family of G protein-coupled receptors.[1] When Wnt is bound to Fzd, the signal is sent to the Dishevelled protein (Dvl) in the cytoplasm, followed by activation of three separate signaling pathways: the canonical (β-catenin-dependent) Wnt pathway, the noncanonical planar cell polarity (Wnt/PCP) pathway, and the Wnt-calcium (Wnt/Ca^{2+}) pathway.[2]

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Conflict of Interest

The authors have no conflicts of interest to declare.

Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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1. The canonical Wnt pathway

The best characterized is the canonical Wnt pathway, which is schematically presented in **Fig. 1**. It governs numerous processes in the body, including cell fate, organogenesis, and bone metabolism. In a resting state, the phosphorylation of β-catenin is controlled by a protein complex consisting of Axin, adenomatous polyposis coli (APC) protein, and glycogen synthase kinase-3 (GSK3). Subsequently, it is tagged by multiple ubiquitin molecules and degraded by proteasomes.[3] When Wnt signaling is active, it triggers Dvl by binding to Fzd receptors and low-density lipoprotein receptor-related protein 1 (LRP1). Dvl then inhibits the function of GSK3 and dismantles the Axin, APC protein, and GSK3 scaffold responsible for β-catenin degradation, leading to its accumulation in the cytoplasm. The β-catenin is then transported to the nucleus, where it is recruited by members of the T-cell factor/lymphoid enhancer-binding factor 1 (LEF1) family and other factors. This recruitment allows the complex to either activate or suppress the target Wnt genes.[4] The fate of the cell is determined by the availability of different receptors. Their interaction with Wnt molecules can lead to the formation of various complexes.[5]

When signaling is inactive, in the absence of Wnt, intracellular β-catenin is phosphorylated by a complex containing Axin, APC, GSK3β, casein kinase (CK1α). Phosphorylated β-catenin undergoes ubiquitination and targeting for proteasomal degradation. Without intranuclear β-catenin, target genes are suppressed via the action of histone deacetylases triggered by the T cell factor (TCF)/LEF proteins. In the active state, Wnt ligands bind Frizzled and LRP

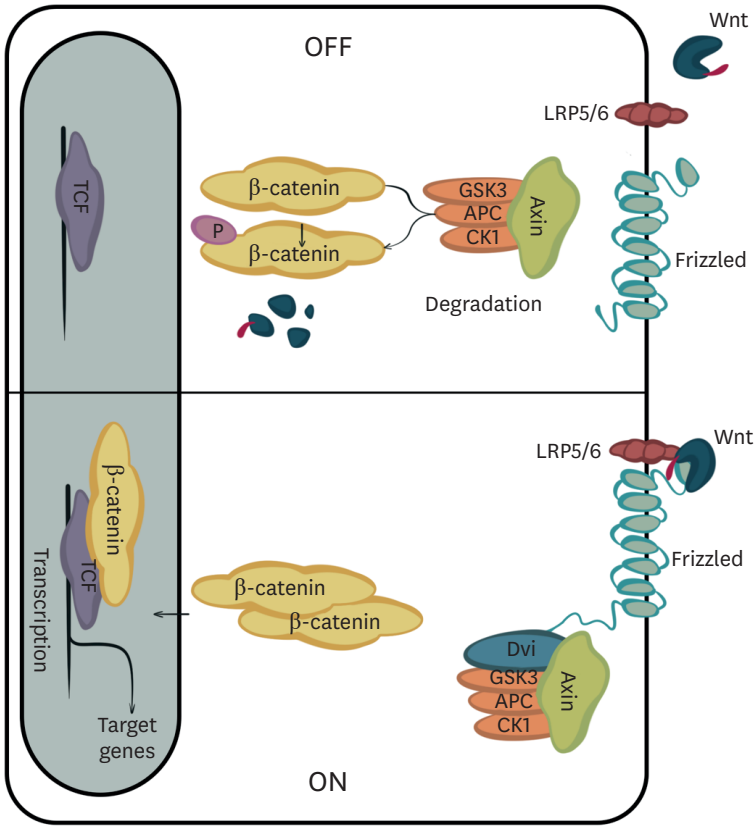

Fig. 1. Canonical Wnt pathway.
TCF, T cell factor; GSK3, glycogen synthase kinase-3; APC, adenomatous polyposis coli; LRP, low-density lipoprotein receptor-related protein.

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co-receptors. This results in LRP phosphorylation by CK1α and GSK3β, and the recruitment of Dvl proteins. Upon accumulation, Dvl become polymerized and activated, inactivating the degradation complex. The cytoplasmic β-catenin is free to accumulate and migrate to the nucleus. In the nucleus, it associates with LEF and TCF and recruits histone-modifying co-activators to promote gene expression and the activation of numerous cellular processes.

2. Noncanonical Wnt pathways

The best-known noncanonical/β-catenin–independent Wnt signaling pathways are Wnt/PCP and Wnt/Ca^{2+}. Other noncanonical pathways involve Ror2, RAP1, PKA, JNK, GSK3MT, and RYK, as well as the Wnt-mTOR pathway.[6] All the above-mentioned pathways overlap to a certain degree. It is believed that Wnt1, Wnt3a, Wnt8, and Wnt8b play a role in the Wnt/β-catenin signaling cascade, while Wnt4 and Wnt5a are involved in the noncanonical cascades. The overlap between the pathways complicates the classification of the molecules.[7]

In the Wnt/PCP pathway, the GTPases Rac and Rho are activated. Since these enzymes regulate alterations in the host cytoskeleton, this pathway can cause lateral asymmetry. In the Wnt/Ca^{2+} pathway, Wnt tethers to the Fzd, stimulating its interaction with the Dvl/Axin/GSK3 complex.[8] GSK3 is involved in the phosphorylation of Ror1 and Ror2, a Wnt-Fzd coreceptor, which results in the activation of phospholipase C, leading to phosphatidylinositol 4,5-bisphosphate cleavage into inositol 1,4,5-triphosphate (IP3) and 1,2-diacylglycerol (DAG). IP3 activates calcium channels on the endoplasmic reticulum (ER), leading to higher levels of Ca^{2+}, which in turn mediates calmodulin-dependent protein kinase II activation.[9] Activated Ca^{2+} particles from the ER also mediate the activation of protein kinase C through 1,2-DAG. The mobilized kinases activate a number of nuclear transcription factors, and the released Ca^{2+} ions may activate the phosphatase calcineurin, a protein susceptible to dephosphorylation, which results in the activation of cytoplasmic nuclear factor related to T cells. The translocation of nuclear factors into the nucleus affects target Wnt gene transcription.[10]

Wnt SIGNALING IN ATHEROSCLEROSIS

The role of Wnt signaling in atherogenesis was reported for the first time in a family from Iran, with autosomal dominant CAD, high blood pressure, high blood lipid levels, and osteoporosis resulting from a missense mutation (R611C) in the *LRP6* gene. Another variant of *LRP6* that is observed more commonly, I1062V, has been associated with a higher risk for atherosclerosis in subjects with high blood pressure.[11] In such individuals, the release of LRP6 in arterial atherosclerotic lesions was much lower; however, unlike R611C, it did not result in hyperlipidemia. Based on the fact that LRP is a co-receptor in the Wnt pathway, these data indicate a possible involvement of this signaling cascade in atherosclerosis. Wnt levels and their implications for atherosclerotic plaques have been examined in several studies.[12] We summarized the potential implications of Wnt signaling to atherosclerosis in **Fig. 2**.

In a study by Christman et al.,[13] higher Wnt5a content was observed in the sites of macrophage accumulation within the intima in plaques of humans and murine models. Additional studies by the same group confirmed the association between Wnt5a content and the degree of atherosclerosis.[14] Wnt5a expression in human macrophages and THP-1 cells could be promoted by oxidized low-density lipoprotein (LDL), while native LDL had no such effect. By contrast, Wnt5a was reported to decrease cellular cholesterol accumulation by moderating the mRNA production of caveolin and ATP-binding cassette transporter A1

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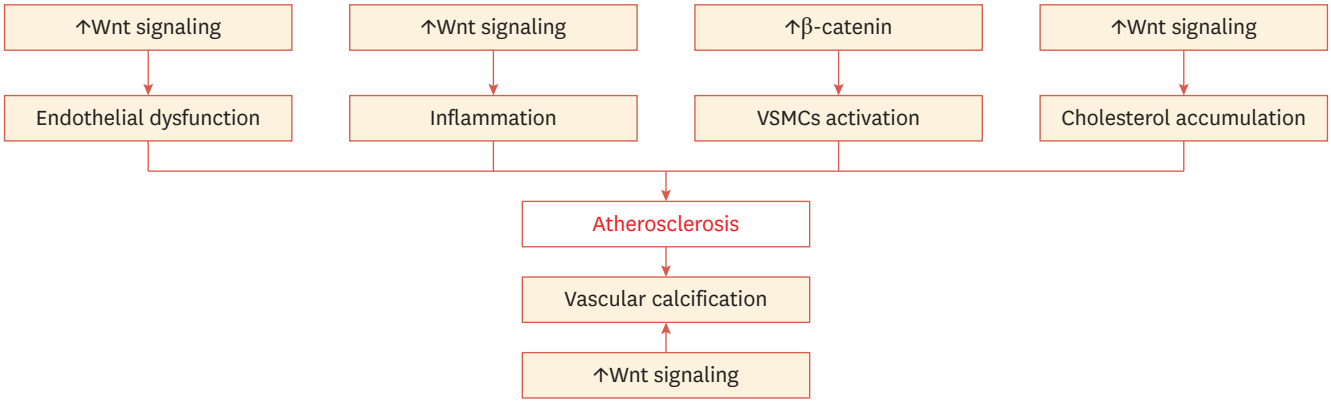

Fig. 2. Scheme of Wnt implication in atherosclerosis.

(ABCA1), which mediate cholesterol efflux. These findings suggest a new pathway for Wnt5a-related cellular cholesterol transport. Wnt-signaling antagonists, such as Dickkopf-related protein 1 (DKK1), were also observed in late-stage atherosclerotic plaques and thrombus material from unstable plaques.

Various molecules involved in the Wnt pathways have been observed not only in plaques, but also in the circulation. Higher Wnt5a and DKK1 levels were reported in patients with atherosclerosis, the latter being also prevalent in subjects with acute myocardial infarction and stroke.[15] DKK1 has also been independently associated with coronary atherosclerosis and calcification and identified as a risk factor for cardiac events. A study of patients with premature myocardial infarction assessed Wnt1 and DKK1 levels 3 days and 12 months following the event.[16] Wnt1 levels were considerably lower than in controls and remained stable throughout the 12-month period, while higher DKK1 concentrations were observed only 12 months after the event. These findings prove that components of Wnt signaling cascades spread over from the impaired tissues to the circulation, where they can easily be detected.[17]

1. Wnt signaling in endothelial dysfunction

Endothelial dysfunction is the first step in atherogenesis. It leads to monocyte recruitment to the subintimal layer of the vessels. There, monocytes transform into macrophages and release cytokines, promoting the accumulation of vascular smooth muscle cells (vSMCs) in the intima.[18] Macrophages and vSMCs lead to lipid retention in the vessel wall, initiating lesion formation. Advanced lesions develop a fibrous cap. Plaque destabilization may lead to a rupture, and result in the formation of a thrombus that can potentially block the artery.[19]

Endothelial cells are a barrier between the blood and the rest of the vessel wall tissue. The endothelium is critically important for blood tone regulation, as it releases blood flow mediators including nitric oxide. Endothelial cells react to both mechanical and chemical triggers.[20] Endothelial damage typically takes place at vessel bifurcation sites where the blood flow is less stable. Endothelial injury triggers the release of pro-inflammatory and coagulation-related genes, leading to the permeation of inflammatory cytokines into the intima. Endothelial dysfunction is an important step in atherogenesis.[21]

Numerous studies have examined the impact of Wnt on endothelial function. Wright et al. identified the presence of certain members of the Fzd and Wnt families in murine human umbilical vein endothelial cell (HUVEC) and brain microvascular endothelial cells. Their

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study noted increased levels of β-catenin due to Wnt1 activity, leading to the mobilization of a (TCF/LEF)-dependent reporter construct, indicative of activity in the canonical Wnt pathway.[22] Elevated levels of Wnt1 were found to stimulate in vitro proliferation of endothelial cells, whereas Wnt5a did not have this effect. However, subsequent studies revealed that Wnt5a/Ca^{2+} signaling encouraged endothelial cell proliferation, while canonical Wnt signaling inhibited it.[23] Goodwin et al.[24] conducted a comprehensive analysis of all Wnt-signaling components present in various types of endothelial cells. They found Wnt2b, 4, and 5a, as well as Fzd6 and 8, in all samples, while the distribution of other Wnt and Fzd varied depending on the origin of the endothelial cells. They also observed that subconfluent endothelial cells exhibited higher levels of intracellular β-catenin stabilization and reporter gene release compared to confluent endothelial cells. The significant variability in the expression of Wnt and Fzd members among endothelial cells of different origins may account for some discrepancies in existing studies.[25]

Both endothelial dysfunction and inflammation play an important role in early atherosclerosis. Members of the Wnt family can influence the proliferation of endothelial cells and mediate related inflammatory processes.[26] Kim et al.[27] reported that endothelial cells, after being exposed to Wnt5a for 1 hour, released inflammatory cytokines such as interleukin (IL)-1α, IL-6, and IL-8, as well as upregulating inflammatory genes such as *COX2*. This expression was even more pronounced with pulsatile exposure. The study's findings suggest that the activation of inflammatory genes results from an interaction between tumor necrosis factor (TNF)-α and Wnt5a, which are responsible for activating nuclear factor (NF)-κB through IKK and Ca^{2+}/protein kinase C (PKC) signaling, respectively.

A recent study evaluated the role of p66shc, a pro-apoptotic protein that mediates oxidative stress, in the canonical Wnt signaling pathway in HUVEC. The study found that Wnt3a rapidly induced the phosphorylation of this protein by JNK at Ser36, a process that could be inhibited by DKK1. When p66shc was knocked down, there was a decrease in β-catenin-related gene transcription. The mobilization of β-catenin reduced the relaxation of aortic rings induced by the endothelium in mice, which was associated with an increase in reactive oxygen species.[28] These findings underscore the significance of p66shc in Wnt3a-induced endothelial dysfunction. Although endothelial cells release components of Wnt signaling pathways, the activation of these signaling cascades could potentially lead to endothelial dysfunction.

2. Wnt signaling in inflammation

Since it is an inflammatory condition, atherosclerosis initiation is to a great extent mediated by macrophages. Monocyte adhesion to endothelial lesions leads to their recruitment into the intima and further differentiation into macrophages. Macrophages accumulate lipids, resulting in foam cell formation. Furthermore, enzymes released by macrophages can weaken the fibrous caps of the plaque, making it less stable. Plaque rupture leads to thrombus formation and may result in acute cardiovascular events.[29]

Studies have shown that areas of macrophage accumulation in lesions are also rich in Wnt5a. This finding indicated a need for further examination of Wnt involvement in macrophage activation. Both bacterial structures and oxidized LDL can promote Wnt5a release in macrophages. Wnt5a and Fzd5 cooperatively initiate the release of inflammatory cytokines, aggravating inflammation.[30] By contrast, Wnt3a inhibits GSK3β, which is responsible for NF-κB-dependent gene transcription, and thus protects against inflammation.[31]

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Borrell–Pages et al. examined the effect of LRP5 on macrophages under lipid overload. Aggregated LDL was used to promote LRP5 transcription.[32] LRP5 suppression by siRNA decreased macrophage lipid retention as well as their motility and resulted in lower LDL uptake. Thus, LRP5 is an important component of the response to lipid permeation and inflammation. The authors also evaluated the effect of LRP5 *in vivo*. The trial involved LRP5 knockout mice on high-lipid feeding. Compared to wild-type controls, the animals gradually developed mild hyperlipidemia and presented with more advanced atherosclerotic lesions, proving the protective role of LRP5 in atherosclerosis. These findings are in line with earlier trials reporting significant foam cell aggregation and aggravated atherosclerosis in apolipoprotein E (apoE)/LRP5 double knockout models in comparison with apoE$^{-/-}$ mice.[33]

3. Wnt signaling in vSMC activation

The recruitment of vSMCs into the intima is an important step in the formation of atherosclerotic plaque. The activation of vSMCs is a complex process, presumably related to inflammation. As a result, vSMCs proliferate and migrate into the arterial wall at the site of the lesion.[34] At the same time their phenotype changes from contractile to synthetic, leading to higher expression of extracellular matrix-related proteins and cytokines and lower expression of contractile proteins.

As evidenced by several studies, β-catenin promotes vSMC proliferation. An element in Wnt signaling, β-catenin enhances the release of genes mediating vSMC proliferation, such as cyclin D1, through TCF4. Several trials using balloon denudation of the carotid artery have confirmed this mechanism. Ligation of the artery in TOPGAL line mice showed that β-catenin-related signaling pathway was active in the intima and media layers on the third and 28th day following the ligation. Furthermore, β-catenin can be found in the cytoplasm, where it is present in its free form.[35] It also occurs at the plasma membrane together with other catenins and cell adhesion molecules, regulating cellular adhesion. When these complexes are dissolved, free β-catenin can be moved to the nucleus and promote vSMC proliferation through the release of D1. This process explains vSMC proliferation upon transfer from the media to the intima.[36]

Several studies have focused on demonstrating Wnt involvement in vSMC proliferation. Those trials have shown that both Wnt1 and Wnt3 can activate the canonical Wnt signaling pathway, leading to the release of cyclin D in vSMCs. It has been proven that the suppression of Wnt signaling through LPR6 significantly reduces vSMC proliferation.[37] Research conducted on mice with inhibited Wnt4 has revealed that this member of the Wnt family also contributes to the endogenous activation of platelet-derived growth factor BB-induced vSMC proliferation. The addition of a Wnt inhibitory factor and the overexpression of sFPR1 have been shown to suppress vSMC proliferation, as demonstrated in both *in vivo* and *in vitro* studies. These findings underscore the significance of Wnt in cell proliferation.[38]

As described above, the inhibition of GSK3β kinase activity, which occurs after the disruption of the Axin, APC protein, and GSK3 scaffold, is a crucial step in the canonical Wnt signaling pathway. The activity of this enzyme can also be suppressed through phosphorylation, which in turn promotes the accumulation of β-catenin. This pathway is implicated in the IL-18-related proliferation of vSMCs in human veins, presumably via an IL-18-induced signaling pathway.[39]

Furthermore, GSK3β also mediates nuclear factor of activated T-cells (NFAT) signaling. Hyperphosphorylated NFAT is found in the cytoplasm, and can be phosphorylated by a

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number of kinases, one of which is GSK3β.[40] The phosphatase calcineurin is activated as a result of exposure to elevated intracellular Ca^{2+} levels. The enzyme dephosphorylates NFAT and triggers its translocation to the nucleus, accelerating vSMC motility and proliferation.[41]

4. Wnt-receptor interaction and cholesterol homeostasis

Cholesterol in mammals is typically synthesized or produced through LDL endocytosis by the LDL receptor (LDLr) and then quickly distributed within the cell through various mechanisms.[42] One involves moving internalized LDL particles to late endosomes, where they are degraded, and esterified cholesterol is then hydrolyzed by acid lipase into free cholesterol. It is then moved to the ER and re-esterified into cholesteryl esters by acyl-coenzyme A (CoA): cholesterol acyltransferases. The production of new cholesterol in the ER is strictly controlled based on its concentration.[43]

When cholesterol levels are elevated, the protein embedded in the membrane binds to Insigs in the endoplasmic reticulum. This action restricts the movement of sterol regulatory element-binding proteins into the nucleus, subsequently reducing cholesterol production.[44]

Numerous pathways involved in the development of atherosclerosis have been identified during the last 30 years. Wnt signaling pathways have recently been identified as important contributors to atherogenesis. Their association with the progression of the disease was initially observed in subjects with a mutation on the Wnt co-receptor LRP6.[45] This receptor belongs to the LDLr family. High levels of cholesterol were reported in these subjects, as well as elevated concentrations of triglycerides and glucose during fasting, providing a basis for the development of metabolic syndrome and atherosclerosis.[46]

Trials using murine models of LRP6 loss-of-function mutation have confirmed its association with coronary artery disease. Additionally, modifications to LRP6 can lead to increased cholesterol synthesis and steatosis in the liver. However, administering Wnt3a to these animals normalized liver cholesterol synthesis and regulated cholesterol and triglyceride levels in the blood.[47] Another Wnt co-receptor, LRP5, also exhibits atheroprotective properties. Murine models with compromised LRP5 developed larger lesions compared to the control group. In a similar vein, apoE/LRP5 double knockout mice developed larger and more advanced lesions than apoE knockout models when fed a high-cholesterol diet.[48]

An association was also observed between low levels of Wnt ligands (specifically low Wnt1) in the blood of patients with atherosclerosis and high levels of triglycerides and cholesterol.[49]

LRP1 is a large endocytic chylomicron remnant receptor found in various tissues. It is responsible for hepatic lipid metabolism and interacts with LDLr. Trials involving gene deletion in some tissues have demonstrated the importance of LRP1 in normal vessel functioning. The lack of the gene in macrophages or smooth muscle cells immediately induces atherosclerosis in mice on high-cholesterol feeding.[50]

Foam cells and free cholesterol crystals were observed in the arterial intima of animals with genetic abnormalities. The deletion of *LRP1* in mouse embryonic fibroblasts (MEFs) in combination with adipogenic cocktail treatment did not induce formation of adipocyte-like cells, but resulted in a significant elevation of free cholesterol and cholesterol ester content in the cells compared to embryonic fibroblasts without the mutation.[51]

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The ability of LRP1 to downregulate cholesterol content and its consequent atheroprotective qualities may be because LRP1 acts as a co-receptor of platelet-derived growth factor receptor β and transforming growth factor (TGF)-β in cholesterol clearance. At the same time, LRP1 is involved in many signaling pathways, including Wnt5a, which may result in the suppression of cholesterol retention in the cells.[52] Wnt5a is abundantly expressed in both vSMCs and MEFs in the presence of LRP1. However, none or little Wnt5a was observed in cells lacking the *LRP1* gene. Cholesterol retention was blocked by recombinant Wnt5a treatment, as well as retransfection of the cells with an expression vector encoding Wnt5a.[53]

In mice lacking LRP1, fewer Wnt5a ligands were observed in aortic vSMCs. This condition leads to the maintenance of higher concentrations of Insig-1, which inhibits the transport of sterol-regulatory element-binding protein from the ER to the Golgi. Consequently, there are lower concentrations of 3-hydroxy-3-methylglutaryl (HMG)-CoA synthase and HMG-CoA reductase, leading to a decrease in cholesterol synthesis. Moreover, vSMCs and macrophages deficient in Wnt5 tended to accumulate more free and total cholesterol when exposed to oxidized LDL. However, treatment with recombinant Wnt5a resulted in a reduction of cholesterol content.[54]

Several studies have demonstrated that the expression of Wnt5a is higher in human atherosclerotic lesions. In one particular cohort of atherosclerotic patients, elevated levels of Wnt5a were observed in roughly 50% of the patients, while the remaining 50% exhibited Wnt5a expression levels similar to those of the control group.[55] However, it is worth noting that this observed association does not necessarily suggest a causal role of Wnt5a in atherosclerosis. Such a suggestion would contradict existing evidence that Wnt signaling actually downregulates lipid retention. These findings underscore the need for more in-depth mechanistic studies, particularly those involving genetics, as well as additional research into cholesterol accumulation in the perinuclear area of Wnt5a-knockout cells and the processes that drive Wnt5a's role in cholesterol downregulation.[56]

Furthermore, Wnt5a can contribute to cholesterol clearance. Caveolae—small invaginations in the cell plasma membrane—can be found in various cell types and contain sphingolipids, caveolin and cholesterol.[57] They are also used as scaffolds by various signaling molecules, such as ABCA1, which mediates cholesterol efflux, or TGF-β, which is involved in Wnt5a secretion in human vSMCs. Wnt5a knockdown cholesterol-rich vSMCs have low content of these signaling molecules, which explains the higher content of total and free cholesterol in the cells. Treatment with Wnt5a can reverse these processes in macrophages or vSMCs and decrease cholesterol content.[58] Other trials have demonstrated the involvement of ATP-binding cassette transporter G1 in Wnt5a-regulated cholesterol clearance. Wnt5a activates this protein, which then promotes cholesterol efflux. Therefore, Wnt5a plays a crucial role in cellular cholesterol regulation and clearance.[59]

5. Involvement of Wnt signaling in atherosclerotic vascular calcification

Evidence indicates that vascular calcification in $LDLR^{-/-}$ mice with diabetes is linked to Wnt signaling upregulation mediated by type 2 bone morphogenetic protein. Bone morphogenetic protein 2 has been found in atherosclerotic plaques, specifically in myofibroblasts and endothelial cells. Its expression is driven by various pathogenic stimuli, such as TNF-α, hyperglycemia, and oxidized lipids.[60] Besides, it mediates osteogenic gene expression programs by activating Msx2 protein responsible for craniofacial calcification. Msx2 is a transcription factor that enhances osteogenic differentiation and at the same time

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inhibits lipid accumulation in myofibroblasts. Msx2 increases the expression of multiple Wnt ligands and downregulates DKK1, a potent antagonist of the Wnt pathway, thus stimulating Wnt signaling.[61] In fact, Wnt and Msx2 appear to reciprocally interact. For example, Msx2 promotes TCF7LEF transcription factors (mediators of Wnt signaling), enhances β-catenin translocation to the nucleus, and upregulates a non-canonical ligand Wnt5 and the canonical ligands Wnt3a and Wnt7.[62]

TARGETING STRATEGIES

In theory, non-canonical Wnt signaling could potentially be chemically inhibited using agents such as Rac family small GTPase 1 (RAC1) or secreted frizzled related proteins (SFRPs). Recent evidence indicates that non-canonical Wnt signaling can be effectively targeted through gene editing techniques, such as CRISPR–Cas9, antisense oligonucleotides (ASOs), and monoclonal antibodies (MAs). However, MAs carry a higher risk of serious adverse events due to their systemic delivery and the resulting inhibition of Wnt in all body tissues.[63] While ASOs and gene editing offer more site-specific inhibition of Wnt signaling, these methods have not yet been incorporated into clinical practice as they require further validation and development. The use of nanoparticles, whether introduced locally or systemically, can enhance the delivery of medications to their intended targets. Wnt signaling could potentially be inhibited effectively and specifically using nanoparticles that are affined to certain molecules, such as endothelial markers, in conjunction with ASOs or gene editing techniques. This approach has been demonstrated in a study where lipid nanoparticles carrying macrophage-specific, promoter-driven plasmids were used to target macrophages causing vessel inflammation.[64]

1. Potential targets

The non-canonical Wnt signaling system, with its complex network of upstream and downstream signal transduction pathways, could theoretically be targeted at various points using the methods previously discussed. However, it should be noted that there is cross-regulation between canonical and non-canonical Wnt signaling. Therefore, any interference with non-canonical Wnt signaling could potentially affect canonical Wnt signaling.[65]

Wnt ligands and SFRP inhibitors

Non-canonical Wnt ligands appear to be the most logical point for therapeutic intervention in the downstream pathway. However, it is not feasible to target all non-canonical ligands simultaneously, so research teams have selected Wnt5a as a representative ligand. Systemic targeting of Wnt5a with MAs or recombinant SFRPs could potentially address this issue. However, as previously noted, this approach carries the risk of inhibiting Wnt in all body tissues. Consequently, it seems more reasonable to target specific tissues, such as body fat tissue, although this is currently not practicable.[65]

Wnt receptors

Non-canonical Wnt signaling could potentially be significantly suppressed by reducing the quantity of Frizzled receptors, such as Fzd2 and Fzd5. These receptors have been observed to be overexpressed in the breast arteries of patients with obesity, suggesting an active interaction with Wnt ligands. The downregulation of these receptors could be achieved through gene silencing or editing technologies, utilizing promoters that are active only in specific cell types. Nevertheless, further research in this field is required.[66]

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Downstream signal transduction molecules

The detrimental effects of noncanonical Wnt signaling could potentially be mitigated by targeting downstream molecules such as calcium/calmodulin-dependent protein kinase II (CaMKII), JNK, RAC1, PKC, and ubiquitin-specific protease 17 (USP17), a recently identified Wnt5a ligand target.[67] However, the challenge lies in the fact that many pathways unrelated to Wnt also utilize these molecules. Therefore, specifically targeting them could result in unintended consequences.

Positive outcomes were observed in mouse models when RAC1 levels were reduced, including a decrease in cardiac oxidative stress and ER stress. This can be achieved using RAC1 allosteric inhibitors. The therapeutic effects of various JNK inhibitors have been identified in animal studies on neurodegeneration and in a phase 1b clinical trial involving humans with lung fibrosis.[68] *In vivo* studies have shown that targeting CaMKII can reduce plaque burden in an apoE knockout mouse model. Another study involving mice reported beneficial effects from using KN93 to target CaMKII in heart failure induced by pressure overload. However, inhibiting PKC *in vivo* presents a challenge due to its numerous isoforms. Recent studies have shown that USP17 enhances RAC1 activation and is a redox-sensitive target downstream of Wnt5a, suggesting it could potentially serve as a therapeutic target.[68]

CONCLUSION

In this review, we have examined the role of Wnt signaling in the onset and progression of atherosclerosis. Numerous studies have investigated the role of various Wnt signaling pathways and their components in the progression of the disease. While there is currently no consensus on the involvement of Wnt in disease progression, it is evident that many Wnt signaling components play a part in the mechanisms of atherogenesis, either directly or by mediating atherogenic processes such as cholesterol accumulation. Research has indicated that mutations in the genes that regulate the secretion of LRP co-receptors can lead to elevated cholesterol levels. Additionally, disordered Wnt function has been found to cause endothelial dysfunction. Furthermore, several studies have corroborated the impact of Wnts on the proliferation and motility of macrophages and vSMCs, ultimately leading to vascular inflammation. Undoubtedly, Wnt signaling can be considered a potential target for the treatment and prevention of atherosclerosis, but the creation of viable therapeutic strategies certainly necessitates further investigation. The main challenge lies in identifying tissue-specific treatment approaches, as a global intervention could potentially have adverse effects on the entire body. Thus, we strengthen the importance of Wnt signaling in the development of atherosclerosis in this review.

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